AMENDMENT – IN THE SPECIFICATION

In addition to the aforementioned amendments to the claims, Applicant also respectfully requests the voluntary correction of some obvious errors in the Specification in accordance with MPEP 714 (II. B). Applicant respectfully requests entry of the following amendments:

1. Vector Name Clarification (pg 7, ln 7-8)

Applicant has noticed some inconsistencies with the vector labels in the specification and respectfully requests entry of the following amendment to bring consistency and easy reference to the various plasmid vectors herein. One important clarification is that the pssXG vector is used as a shorthand method to refer to different forms of the pssXG vectors in several places in the specification. It is important to note that in this specification, there is no pssXG vector described that does not have an addendum in its name. In this disclosure, only the pssXGa, pssXGb and pssXGb(FtsZ-DZ) vectors are described or presented. The pssXG vector designation was mistakenly (and confusingly) used where more lengthy names should have been used. Applicant therefore requests correction of the references to the pssXG vector with the appropriate names and argues that one reasonably skilled in the art would readily deduce these corrective names using the original disclosure alone, and therefore argues that this would not constitute new matter.

To make the disclosure easier to understand, Applicant respectfully requests that the following vector designation be changed in accordance with the following:

pg 7, ln 8	pssXG	should be	pssXGa
pg 10, ln 9	pssXG	should be	pssXGb(FtsZ-DZ)

The first of these corrections follows here:

Please replace:

> Figure 1 is a schematic representation of a preferred embodiment of the expression vector pssXGa constructed in accordance with the teachings of the present invention.

With (new):

> Figure 1 is a schematic representation of a preferred embodiment of the expression vector pssXGa constructed in accordance with the teachings of the present invention.

2. Obvious Error (pg 8, ln 26-30)

The application as originally filed incorporates by reference International Application PCT/US00/27381 as support for construction of a parent vector (pssXE) that is modified in the current invention to make the ssDNA expression vector (pssXGb) which is in turn used for cloning and expression of the random sequences of the claimed bacterial library.

Applicant recognizes that the PCT reference is in error and requests that to avoid confusion, the incorporation by reference to this PCT be deleted at this particular point in the

specification and replaced with a mere "reference" to the correct prior application (note, this PCT and its original incorporation by reference is maintained later at page 10, line 29; see amendment 7, below). Applicant respectfully argues that since the replaced reference will not be incorporating any new matter other than the identifier of the proper reference application, this amendment should be treated solely as an obvious mistake. The publication date of this U.S. Patent Publication is May 1, 2002, occurring prior to the earliest priority date claimed in the current application, June 3, 2003. As such, Applicant requests entry of the following amendment:

Please replace:

Construction of tetracycline-inducible prokaryotic ssDNA expression vector.

PCR amplification was carried out using pssXE, described in ~~International Application No. PCT/US00/27381, which application is hereby incorporated into this specification in its entirety by this specific reference,~~ U.S. Patent Application Serial No: 10/136,218 (U.S. Patent Publication 2003/0082800), as the template. DNA primers used in the PCR reaction, 5'NheIPvuIATG

With (new):

Construction of tetracycline-inducible prokaryotic ssDNA expression vector.

PCR amplification was carried out using pssXE, described in U.S. Patent Application Serial No: 10/136,218 (U.S. Patent Publication 2003/0082800), as the template. DNA primers used in the PCR reaction, 5'NheIPvuIATG

3. Clarification and Obvious Errors (pg 9, ln 2-23)

Applicant respectfully requests the following amendments to both clarify and correct obvious errors in compliance with 35 U.S.C. 112, first paragraph, by reorganizing the written description without the addition of new matter, in a manner so that one skilled in the art can more easily understand some of the details of the current invention.

Please replace:

5'-CGGGGTACCAGTATTCCCTGGTC-3' [Seq. ID No. 2]

were synthesized by Integrated DNA Technologies (Coralville, IA). The PCR amplified DNA fragment was double-digested with NheI and KpnI and then ~~subcloned~~ recloned back into the original pssXE vector that was double-digested with the same enzymes. The replacement removes the sequence before the translation starting site (ATG), which is unnecessary for prokaryotic gene expression, while creating a new restriction enzyme site, PvuI. The newly created construct was digested with PvuI and XbaI. The PvuI-XbaI fragment contains all the essential elements for ssDNA production, including: 1) Mouse Moloney leukemia viral reverse transcriptase (MoMuLV RT) gene coding for a truncated but fully active RT (Tanase & Goff, PNAS, 2000, 85:1777-1781); 2) primer binding site (PBS) along with some flanking regions of the promoter that are essential for the reverse transcription initiation by MoMuLV RT (Shinnick, *et al.*, Nature, 1981, 293:543-548); and 3) a stem-loop structure designed for the termination of the reverse transcription reaction, all as described in the above-referenced ~~incorporated International Application No. PCT/US00/27381~~ U.S. Patent Application Serial No: 10/136,218. This DNA fragment was

subcloned into the pPROTet.E 233 vector (BD Bioscience, Palo Alto, CA) and the newly created construct was designated as pssXGa, shown in Fig. 1. ~~However, t~~ The sequence of bacteria tRNAPro is different from mammalian tRNAPro, which was ~~designed to bind with the PBS~~ used in the pssXE vector for expression in mammalian cells. ~~Because~~ Bacterial tRNAVal can be utilized as a primer for RT in bacterial cells, so ~~a new PBS was designed to replace~~ the mammalian PBS ~~used in the vector pssXE that is used for mammalian cells.~~ was replaced with the bacterial tRNAVal in vector pssXGb. The sequence of the novel PBS is

5'-TGGTGCGTCCGAG-3' [Seq. ID No. 3].

With (new):

5'-CGGGGTACCAGTATTCCCTGGTC-3' [Seq. ID No. 2]

were synthesized by Integrated DNA Technologies (Coralville, IA). The PCR amplified DNA fragment was double-digested with NheI and KpnI and then recloned back into the original pssXE vector that was double-digested with the same enzymes. The replacement removes the sequence before the translation starting site (ATG), which is unnecessary for prokaryotic gene expression, while creating a new restriction enzyme site, PvuI. The newly created construct was digested with PvuI and XbaI. The PvuI-XbaI fragment contains all the essential elements for ssDNA production, including: 1) Mouse Moloney leukemia viral reverse transcriptase (MoMuLV RT) gene coding for a truncated but fully active RT (Tanase & Goff, PNAS, 2000, 85:1777-1781); 2) primer binding site (PBS) along with some flanking regions of the promoter that are essential for the reverse transcription initiation by MoMuLV RT (Shinnick, *et al.*, Nature, 1981, 293:543-548); and 3) a stem-loop structure designed for the termination of the reverse transcription reaction, all as described in the above-referenced U.S. Patent Application Serial No: 10/136,218. This DNA fragment was subcloned into the pPROTet.E 233 vector (BD Bioscience, Palo Alto, CA) and the newly created construct was designated as pssXGa, shown in Fig. 1. The sequence of bacteria tRNAPro is different from mammalian tRNAPro, which was used in the pssXE vector for expression in mammalian cells. Bacterial tRNAVal can be utilized as a primer for RT in bacterial cells, so the mammalian PBS was replaced with the bacterial tRNAVal in vector pssXGb. The sequence of the novel PBS is

5'-TGGTGCGTCCGAG-3' [Seq. ID No. 3].

4. Clarification (pg 10, ln 4-19)

Please replace:

pPROTet.E233 is a tetracycline-inducible bacterial expression vector expressing fusion protein with the 6xHN histidine tag sequence: His-Asn-His-Asn-His-Asn-His-Asn-His-Asn-His-Asn (SEQ ID NO: 33). It utilizes a novel promoter, $P_{Ltet}O1$, which is tightly repressed by the highly specific Tet repressor protein and induced in response to anhydrotetracycline (aTc), allowing control of induction over a wide ~~range (anhydrotetracycline~~ range. Anhydrotetracycline is a derivative of tetracycline that acts as a more potent inducer of PROTet.E ~~Systems).~~ Systems. The pssXG(FtsZ-DZ) vector was transformed into the bacteria strain, DH5αPro (BD Bioscience, Palo Alto, CA) in the presence of 34 μg/ml choloramphenicol (Cm) and 50 μg/ml spectinomycin (spec). Spectinomycin is used to select for DH5αPro cells that carry transcription units encoding TetR (Lutz & Bujard, Nucleic

Acids Res., 1997, 25:1203-1210). The DH5αPro cells express defined amounts of the Tet repressors. Cell lysates were prepared using B-PER II Bacterial Protein Extraction Reagent (Pierce, Rockford, IL) according to the manufacturer's instruction. Using the cell lysates, the expression of reverse transcriptase (RT) was confirmed by RT activity assay using cell lysates according to Silver, *et al.* (Nucleic Acids Res., 1993, 21:3593-3594) as shown in Fig. 3 and Western blotting using antibody against 6xHN (SEQ. ID NO: 33) (BD Bioscience, Palo Alto, CA) as shown in Fig. 4.

With (new):

pPROTet.E233 is a tetracycline-inducible bacterial expression vector expressing fusion protein with the 6xHN histidine tag sequence: His-Asn-His-Asn-His-Asn-His-Asn-His-Asn-His-Asn (SEQ ID NO: 33). It utilizes a novel promoter, $P_{Ltet}O1$, which is tightly repressed by the highly specific Tet repressor protein and induced in response to anhydrotetracycline (aTc), allowing control of induction over a wide range. Anhydrotetracycline is a derivative of tetracycline that acts as a more potent inducer of PROTet.E Systems. The pssXG(FtsZ-DZ) vector was transformed into the bacteria strain, DH5αPro (BD Bioscience, Palo Alto, CA) in the presence of 34 μg/ml choloramphenicol (Cm) and 50 μg/ml spectinomycin (spec). Spectinomycin is used to select for DH5αPro cells that carry transcription units encoding TetR (Lutz & Bujard, Nucleic Acids Res., 1997, 25:1203-1210). The DH5αPro cells express defined amounts of the Tet repressors. Cell lysates were prepared using B-PER II Bacterial Protein Extraction Reagent (Pierce, Rockford, IL) according to the manufacturer's instruction. Using the cell lysates, the expression of reverse transcriptase (RT) was confirmed by RT activity assay using cell lysates according to Silver, *et al.* (Nucleic Acids Res., 1993, 21:3593-3594) as shown in Fig. 3 and Western blotting using antibody against 6xHN (SEQ ID NO: 33) (BD Bioscience, Palo Alto, CA) as shown in Fig. 4.

5. Obvious Error (pg 10, ln 29-30)

Applicant requests the Examiner to refer to Amendment no. 2, above to confirm that the below requested amendment is simply a correction and does not involve the addition of new matter.

Please replace:

Because of their ability to bind and cleave any target RNA at purine/pyrimidine junctions, DNA enzymes are capable of interfering with gene expression as described in ~~the above incorporated~~ International Application No. PCT/US00/27381, which application is hereby incorporated by reference in its entirety.

With (new):

Because of their ability to bind and cleave any target RNA at purine/pyrimidine junctions, DNA enzymes are capable of interfering with gene expression as described in International Application No. PCT/US00/27381, which application is hereby incorporated by reference in its entirety.

6. Addition of Flow Chart of Important Aspects of the Invention (pg 7, ln 6)

Although this is not needed, Applicant argues that by pulling excerpts of the current disclosure out and compiling them in a unified flow chart, the important steps of the invention will be emphasized. As each point is supported by the disclosure as filed, Applicant argues that this would not constitute new matter. Applicant notes that the term screening as used herein as described on page 13 of the original specification. Screening in this context means a series of steps including plating of bacteria for single colonies on nutrient plates, replica plating on nutrient and inducer plates (tetracycline or anhydrotetracycline (aTc)) to induce expression of the ssDNA, and identifying colonies unable to grow when the inducer is present.

It is requested that the following flow diagram be inserted into the text at the end of the section labeled Summary of the Invention on page 7:

Please Insert (new):

Construction and Use of the Prokaryotic ssDNA Expression Vector

(1) construct a useful ssDNA expression vector (pssXGb) for expression of ssDNA oligos inside a bacterial cell - involves cloning of the ssDNA expression cassette of pssXE into a bacterial tetracycline inducible vector and replacing the mammalian expression system PBS of pssXE with a PBS sequence preferred by bacterial expression systems;
(2) construct a bacterial ssDNA expression library useful for identifying sequences that can halt or stop bacterial cell growth - involves the cloning of random sequences (with or without the inclusion of a DNA enzyme sequence) into the bacterial ssDNA expression vector;
(3) screen the bacterial ssDNA expression library to identify useful bacterial growth-altering sequences; and
(4) characterize the identified sequences as potential antimicrobial agents when induced to express the ssDNA inside the bacterial cell.